 doi 10.3969/j.issn.1673-4254.2015.05.21 J South Med Univ, 2015, 35(5): 728-732

临床研究

急性髓系白血病CDX2和β-catenin基因表达的相关性

王 巍[1,2],孟 灿[1,2],刘 佳[1,2],杨志刚[1,2]
广东医学院[1]血液病研究室,[2]附属医院血液内科,广东 湛江 524000

摘要:目的 研究急性髓系白血病(AML)患者CDX2与β-catenin基因表达的相关性。**方法** 实时定量PCR方法检测92例初诊AML、30例非恶性血液病患者(对照)CDX2及β-catenin mRNA表达,Western blot检测其中的26例AML、8例非恶性血液病患者CDX2、β-catenin蛋白表达。**结果** AML组CDX2 mRNA和蛋白表达阳性率分别为84.78%和76.92%,对照组未检测到表达(P<0.01);β-catenin mRNA和蛋白在两组均表达,但AML组显著高于对照组(P<0.01)。CDX2、β-catenin mRNA表达均与初诊时白细胞数、LDH水平呈正相关(P<0.01)。AML患者获得完全缓解时,CDX2 mRNA转阴、β-catenin mRNA表达显著下降,复发时两者均增高。AML患者CDX2与β-catenin无论在mRNA和蛋白水平表达均呈显著正相关(P<0.01)。**结论** AML患者存在CDX2基因过表达及Wnt/β-catenin信号通路激活,且CDX2与β-catenin表达呈显著正相关。
关键词:急性髓系白血病;CDX2;β-catenin;基因表达

Expressions of CDX2 and β-catenin and their correlation in acute myeloid leukemia

WANG Wei[1,2], MENG Can[1,2], LIU Jia[1,2], YANG Zhigang[1,2]
Laboratory of Hematology[1], Department of Hematology, Affiliated Hospital[2], Guangdong Medical University, Zhanjiang 524000, China

Abstract: Objective To investigate the expressions of CDX2 and β-catenin and their correlation in acute myeloid leukemia (AML). **Methods** Real-time PCR was used to examine the expressions of CDX2 and β-catenin mRNA in 92 de novo AML and 30 non-malignant patients (control), and Western blot was employed to detect CDX2 and β-catenin protein expressions in 26 de novo AML and 8 non-malignant patients. **Results** The positivity rates of CDX2 mRNA and protein expressions in AML group were 84.78% and 76.92%, respectively, but no CDX2 mRNA or protein expression were detected in control group (P< 0.01). The expressions of β-catenin mRNA and protein were detected in all the patients in both groups but its expressions in AML group were significantly higher (P<0.01). The expressions of CDX2 and β-catenin mRNA were significantly correlated with WBC counts and LDH levels (P<0.01). The expression of CDX2 mRNA was not detected and the expression of β-catenin mRNA was decreased to normal level in AML patients with complete remission, while the expressions of CDX2 and β-catenin mRNA were increased again in those with disease relapse. There were significantly positive correlations between CDX2 and β-catenin expressions at both mRNA and protein levels (P<0.01). **Conclusion** Up-regulation of CDX2 gene and activation of Wnt/β-catenin pathway co-exist in AML patients and the expressions of CDX2 and β-catenin are positively correlated.
Key words: acute myeloid leukemia; CDX2; β-catenin; gene expression

CDX2基因(cadual type homeobox gene-2,尾型同源盒-2)属于同源盒基因(HOX)家族中的一员,是发育相关基因,其表达失调与结直肠癌、急性白血病发病关系密切[1-4],在急性白血病中CDX2的主要作用是促进细胞增殖、增强克隆形成能力[4-5]。Wnt/β-catenin信号通路是一条在进化中较为保守的通路,参与胚胎发育调节,其异常激活与多种肿瘤发病相关,如结直肠癌、白血病等[6-7],该信号通路在急性白血病中也具有促进细胞增殖的作用[8-9]。CDX2基因和Wnt/β-catenin信号通路在急性白血病中的功能有相似之处,两者是否有协同作用?β-catenin是Wnt/β-catenin信号通路的关键分子,其表达增高可反映该通路的激活,为研究急性髓系白血病(AML)CDX2表达上调与Wnt/β-catenin信号通路激活的相关性,本文对CDX2和β-catenin基因从表达水平、临床意义、与疗效关系及不同病程表达变化几个方面进行了分析。

收稿日期:2014-12-05
基金项目:广东省自然科学基金(S2012040006822);广东省医学科研基金(A2012419);东莞市高等院校、科研机构科技计划项目(2012108102041);广东医学院博士启动基金(XB1017);湛江市科技攻关项目(2010C3104010)
作者简介:王 巍,副教授,E-mail: 532723336@qq.com;孟 灿,硕士,E-mail: 564680017@qq.com。王 巍、孟 灿共同为第一作者
通信作者:杨志刚,教授,博士生导师,E-mail: yangzhigangcn@aliyun.com

1 材料与方法

1.1 病例资料

收集我科2011年12月~2013年12月期间诊治的初发AML患者骨髓标本92例,诊断符合张之南主编的《血液病诊断及疗效标准》。其中男性54例、女性38例,中位年龄48岁(9~87岁)。按照FAB分型:M1型1例、

M2型20例、M3型9例、M4型25例、M5型22例、M6型3例、AML未定型12例。诱导治疗非M3患者采用标准剂量DA(柔红霉素+阿糖胞苷)、DAE(柔红霉素+阿糖胞苷+依托泊苷)、CAG(吡柔比星+阿糖胞苷)、IA(去甲氧柔红霉素+阿糖胞苷)方案;M3患者予以全反式维甲酸联合DA方案化疗。对照组30例为同期诊治的非恶性血液病患者(增生性贫血21例,特发性血小板减少性紫癜9例),其中男性16例、女性14例,中位年龄42岁(15~82岁)。

1.2 主要试剂和仪器

TRIzol、逆转录试剂盒、SYBR®Premix Ex Taq™II试剂盒均购自TaKaRa公司,兔抗人CDX2抗体购自美国Abcam公司(ab76541)、兔抗人β-catenin抗体购自美国Proteintech公司(#51067-2-AP)、兔抗人GAPDH抗体购自Proteintech公司(#10494-1-AP),BCA蛋白定量及ECL发光试剂盒购自碧云天公司,化学发光成像系统为美国Proteinsimple公司。

1.3 方法

1.3.1 骨髓单个核细胞分离 取抗凝骨髓液2~5 ml,淋巴细胞分离液分离单个核细胞,加TRIzol或蛋白裂解液提取RNA或蛋白。

1.3.2 实时定量PCR 1×10^7细胞加1 ml TRIzol吹打混匀提取总RNA,紫外分光光度计检测RNA质量和浓度,按照逆转录酶试剂盒说明书中的步骤逆转录cDNA。PCR引物设计好后由广州Invitrogen公司合成,CDX2上游:5'-TTCACTACAGTCGCTACATCACC-3',下游:5'-GCTGCAACTTCTTCTTGTTGATT-3',扩增片段141 bp;β-catenin上游:5'-TGCAGTTCGCCTTCACTATG-3',下游:5'-ACAGTCGTGGAATGGCACC-3',扩增片段162 bp;内参基因GAPDH上游:5'-GTCAGTGGTGGACCTGACCT-3',下游:5'-TGAGCTTGACAAAGTGGTCG-3',扩增片段212 bp;。按SYBR®Premix Ex Taq™ II试剂盒说明书进行PCR反应,总体系10 μl,其中SYBR®Premix Ex Taq II(2×)5 μl,上下游引物(10 μmol/L)各0.4 μl,cDNA 1 μl,灭菌蒸馏水3.2 μl。PCR扩增条件:95 ℃预变性30 s,95 ℃ 5 s,60 ℃ 30 s,共45个循环。计算$2^{-\Delta CT}$值作为各标本mRNA相对表达量,-ΔCT=(目的基因CT值-内参基因CT值)。

1.3.3 Western blot 取1×10^7细胞,PBS洗涤后悬浮于RIPA裂解液冰上裂解30 min,10 000 r/min离心10 min后取上清,BCA法测定蛋白浓度。取50~70 μg总蛋白进行聚丙烯酰胺凝胶电泳,电转至PVDF膜。5%脱脂奶封闭90 min,加入抗CDX2(1∶500稀释)、β-catenin(1∶1000稀释)、GAPDH(1∶1000稀释)一抗4 ℃孵育过夜,TBST洗涤后室温孵育二抗2 h,TBST再次洗涤后ECL法显色。蛋白相对表达量=灰度值(母的蛋白)/灰度值(内参蛋白)。

1.4 统计学处理

采用SPSS17.0软件进行统计学分析。样本不符合正态分布,组间比较用Mann-Whitney U检验,AML各亚型整体比较用Kruskal-Wallis H检验,相关性分析用Spearman相关,高表达与低表达组间完全缓解率比较用χ^2检验,初诊与治疗后的比较用配对的非参数分析。$P<0.05$认为有统计学差异。

2 结果

2.1 RNA质量及方法可靠性分析

所提RNA经分光光度计检测,A_{260nm}/A_{280nm}值均在1.8~2.0之间,纯度好,可用于定量PCR检测。熔解曲线分析显示,各基因PCR扩增产物熔解温度均一,熔解峰为锐利的单一峰,说明扩增产物均一,无非特异扩增及引物二聚体。

2.2 CDX2、β-catenin基因在AML中的表达

定量PCR法检测92例初发AML和30例非恶性血液病患者(对照)CDX2和β-catenin表达,CDX2 mRNA在AML组的阳性率为84.78%,对照组无1例者表达(Z=-7.125,P=0.000)。β-catenin mRNA在两组均有表达,但AML组显著高于对照组(Z=-6.222,P=0.001)。CDX2和β-catenin mRNA表达水平在AML各亚型间(除外M1)均无统计学差异(P<0.05,表1)。

收集到初发AML患者蛋白标本26例,非恶性血液病患者8例。Western blot检测显示,AML组有20例标本可检测到CDX2蛋白表达,阳性率为76.92%,对照组未检测到CDX2蛋白表达(Z=-4.103,P=0.001)。β-catenin蛋白在两组均有表达,AML组显著于对照组(Z=-3.662,P=0.001,表2、图1)。β-catenin、CDX2在蛋白水平的表达趋势基本与mRNA水平一致。

2.3 CDX2、β-catenin mRNA表达与AML患者临床特征的关系

CDX2 mRNA表达与AML临床特征的关系基本与β-catenin一致,两者均与AML患者性别、年龄、骨髓幼稚细胞比例无显著性相关,与初诊时白细胞数、LDH水平呈显著正相关(表3)。这说明CDX2、β-catenin mRNA表达水平能反映白血病肿瘤负荷情况,肿瘤负荷越高,CDX2、β-catenin mRNA表达水平越高。

2.4 CDX2和β-catenin mRNA表达与AML患者疗效的关系及病程中动态观察

可评估疗效的AML患者54例,CDX2 mRNA表达阳性45例,阴性9例。45例阳性表达患者中首疗程完全缓解(complete remission, CR)31例,CR率为68.89%;9例阴性表达患者首疗程CR 7例,CR率为77.78%,两组

表1 CDX2和β-catenin mRNA在AML组和对照组中的表达
Tab.1 Expressions of CDX2 and β-catenin mRNA in AML and control groups

Group		*n*	Median (range)	
			CDX2 (×10⁻⁸)	β-catenin (×10⁻⁴)
Control		30	0	84.92(23.39-238.48)
AML		92	50.34 (0-2475.49)*	248.63(64.34-2348.80)*
	M1	1	256.62	987.55
	M2	20	44.49(0-601.95)	191.79(91.63-980.73)
	M3	9	93.93(0-237.78)	147.82(98.89-529.22)
	M4	25	59.86(0-1138.97)	389.29(79.22-1538.93)
	M5	22	60.03(0-2475.49)	249.65(66.16-2348.81)
	M6	3	4.67(0-18.05)	115.18(94.20-156.25)
	No-known type	12	21.57(0-601.95)	207.93(64.34-1582.20)

*P<0.01 *vs* control group.

表2 CDX2和β-catenin蛋白在AML组和对照组中的表达
Tab.2 Expressions of CDX2 and β-catenin protein in AML and control groups

Group	*n*	Median (range)	
		CDX2	β-catenin
AML	26	0.37(0-0.67)*	0.74(0.31~1.18)*
Control	8	0	0.31(0.19-0.37)

*P<0.01 *vs* control group.

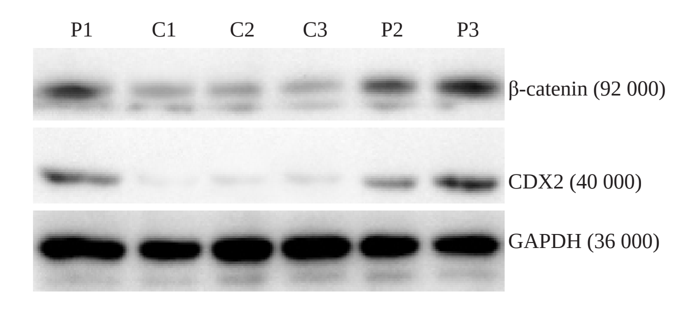


图1 *Western blotting*检测AML组与对照组CDX2、β-catenin蛋白表达
Fig.1 Expressions of CDX2 and β-catenin protein in AML (P) and control (C) groups detected by *Western blotting*.

比较差异无统计学意义(χ^2=0.284,P=0.594)。

配对分析同一AML患者初诊时和首疗程治疗后CDX2和β-catenin mRNA表达变化,共收集到符合标准的AML患者39例,首疗程达CR 23例,未达CR 16例(PR 7例、NR 9例)。患者获CR时CDX2 mRNA下降至检测不到,β-catenin mRNA表达下降至与对照组水平无显著差异(Z=-1.828,P=0.068),而未获CR患者CDX2和β-catenin mRNA表达水平介于初诊和CR时之间(表4)。

跟踪5例首疗程未达CR患者(初诊时CDX2均阳性),第二疗程均达CR,此时CDX2 mRNA表达转阴,β-catenin mRNA表达下降至与对照组水平接近。收集到复发标本2例,这2例患者初诊时CDX2均阳性,CR时CDX2 mRNA表达转阴,β-catenin mRNA表达显著下降,分别于诊断7、10个月后复发,复发时CDX2 mRNA由阴转阳,β-catenin mRNA表达显著升高。

2.5 AML中CDX2与β-catenin表达的相关性分析

Spearman相关分析显示,无论在mRNA水平还是蛋白水平,CDX2与β-catenin表达均呈显著正相关(r=0.793,P=0.001;r=0.804,P=0.001)。

3 讨论

CDX2基因属于尾型同源框基因家族中的一员,是一种转录因子,参与胚胎发育、造血调控等。CDX2最初在胚胎的3个胚层均广泛表达,以后逐渐集中在内胚层表达,从新生儿早期开始至整个成年期基本只表达于小肠和结肠,调控肠道组织发育[10]。CDX2基因表达下调与肠道肿瘤密切相关[1-2],被认为是肠道肿瘤的抑癌基因[11]。近年来的研究发现,急性白血病患者可检测到CDX2的异常表达,CDX2具有促进细胞增殖和克隆形成的作用,并可直接诱发小鼠AML[4-5]。由此可见,CDX2基因在不同细胞背景下功能不同[12-13],其在肠道肿瘤发病中起的是抑癌基因作用,而在AML发病中起的是原癌基因作用。Wnt/β-catenin通路是体内重要的信号转导通路,其异常激活与白血病发病相关。

为研究CDX2表达上调与Wnt/β-catenin信号通路异常激活的相关性,本文分析了AML患者CDX2和β-catenin基因的表达、临床意义、与疗效的关系,并观察了两基因在AML不同病程的表达变化。结果显示,84.78%的AML患者表达CDX2 mRNA,而非恶性血液病对照无1例表达,与黎国伟等[14]报道的基本一致;同时

表3 CDX2、β-catenin mRNA表达与AML临床特征的关系

Tab.3 Correlations of CDX2 and β-catenin mRNA expressions with clinical characteristics in AML patients

Clinical parameter	Median(range)	CDX2		β-catenin	
		r	*P*	*r*	*P*
Age	48 (9-87)	0.090	0.392	-0.079	0.471
WBC count ($\times10^9$/L)	36 (1.3-401)	0.641	0.001	0.576	0.001
Platelet ($\times10^{12}$/L)	38 (7-228)	0.118	0.514	-0.159	0.378
Hemoglobin (g/L)	67 (26-145)	0.225	0.152	0.338	0.055
LDH (U/L)	443.7 (117-3137.9)	0.563	0.001	0.367	0.001
Blasts in BM (%)	63.25 (20-96.5)	0.185	0.077	0.171	0.102

表4 初诊、首疗程治疗后及对照组CDX2、β-catenin mRNA表达水平比较

Tab.4 Comparison of CDX2 and β-catenin mRNA expressions among new diagnosis, after first chemotherapy and control groups

Group		*n*	Median (range)	
			CDX2 ($\times10^{-8}$)	β-catenin ($\times10^{-4}$)
CR	New diagnosis	23	16.72 (0.00-2475.49)	128.69 (79.26-2348.81)
	After first chemotherapy	23	0*	87.89 (16.09-193.70)*#
No CR	New diagnosis	16	53.87 (0.00-1138.97)	330.55 (91.63-1538.93)
	After first chemotherapy	16	25.98 (0.00-141.39)	187.76 (71.89-551.69)
Control		30	0	84.92 (23.39-238.48)

*P<0.01 *vs* new diagnosis at the same group; #P>0.05 *vs* control group.

AML患者有β-catenin的过度表达，且CDX2与β-catenin无论在mRNA还是蛋白表达水平均呈显著正相关。在临床意义方面，CDX2与β-catenin基本相同，两者均与反映肿瘤负荷的指标如初诊时白细胞数、LDH水平呈正相关，而与性别、年龄、骨髓幼稚细胞比例无显著性相关。在基因表达与AML疗效方面，CDX2基因表达阳性与否对首疗程CR率无显著影响。CDX2与β-catenin表达的动态变化均可反映疾病进程，当患者获得CR时，CDX2表达转阴、β-catenin下降至正常水平，复发时CDX2、β-catenin表达再次增高。CDX2与β-catenin基因在AML中的临床意义、不同病程表达变化基本一致，这也从另一个方面反映了CDX2与β-catenin之间存在相关性。两者之间究竟是怎样一种联系呢？查阅文献发现，在爪蟾胚胎和结肠腺癌细胞，Wnt/β-catenin信号通路调控CDX2表达[15-16]，AML细胞中CDX2基因表达上调是否是由Wnt/β-catenin异常激活引起的是我们下一步要研究的重点。

另外，绝大部分AML患者表达CDX2基因，且CDX2表达在各亚型间无显著差异，提示CDX2基因有可能成为广谱的白血病标志物。正常人和非恶性血液病患者不表达CDX2基因，CDX2基因仅在白血病细胞表达，且其表达水平与肿瘤负荷密切相关，并随疾病病程呈动态变化，提示CDX2基因有可能成为监测白血病微小残留的指标之一，但尚需扩大标本量、定期检测CDX2表达(如每隔3个月)、延长跟踪时间进一步观察。

参考文献：

[1] Werling RW, Yaziji H, Bacchi CE, et al. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas[J]. Am J Surg Pathol, 2003, 27(3): 303-10.

[2] Hinoi T, Tani M, Lucas PC, et al. Loss of CDX2 expression and microsatellite instability are prominent features of large cell minimally differentiated carcinomas of the colon[J]. Am J Pathol, 2001, 159(6): 2239-48.

[3] Thoene S, Rawat VP, Heilmeier B, et al. The homeobox gene CDX2 is aberrantly expressed and associated with an inferior prognosis in patients with acute lymphoblastic leukemia[J]. Leukemia, 2009, 23 (4): 649-55.

[4] Scholl C, Bansal D, Döhner K, et al. The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis[J]. J Clin Invest, 2007, 117(4): 1037-48.

[5] Rawat VP, Thoene S, Naidu VM, et al. Overexpression of CDX2 perturbs HOX gene expression in murine progenitors depending on its N-terminal domain and is closely correlated with deregulated HOX gene expression in human acute myeloid leukemia[J]. Blood, 2008, 111(1): 309-19.

[6] Smith K, Bui TD, Poulsom R, et al. Up-regulation of macrophage wnt gene expression in adenoma-carcinoma progression of human colorectal cancer[J]. Br J Cancer, 1999, 81(3): 496-502.

[7] Simon M, Grandage VL, Linch DC, et al. Constitutive activation of the Wnt/beta-catenin signalling pathway in acute myeloid leukaemia [J]. Oncogene, 2005, 24(14): 2410-20.

[8] Chung EJ, Hwang SG, Nguyen P, et al. Regulation of leukemic cell adhesion, proliferation, and survival by beta-catenin [J]. Blood, 2002, 100(3): 982-90.

[9] Ysebaert L, Chicanne G, Demur C, et al. Expression of beta-catenin by acute myeloid leukemia cells predicts enhanced clonogenic capacities and poor prognosis[J]. Leukemia, 2006, 20(7): 1211-6.

[10] James R, Erler T, Kazenwadel J. Structure of the murine homeobox gene cdx-2. Expression in embryonic and adult intestinal epithelium [J]. J Biol Chem, 1994, 269(21): 15229-37.

[11] Bonhomme C, Duluc I, Martin E, et al. The Cdx2 homeobox gene has a tumour suppressor function in the distal colon in addition to a homeotic role during gut development [J]. Gut, 2003, 52(10): 1465-71.

[12] Renouf B, Soret C, Saandi T, et al. Cdx2 homeoprotein inhibits non-homologous end joining in colon cancer but not in leukemia cells[J]. Nucleic Acids Res, 2012, 40(8): 3456-69.

[13] Faber K, Bullinger L, Ragu C, et al. CDX2-driven leukemogenesis involves KLF4 repression and deregulated PPARγ signaling [J]. J Clin Invest, 2013, 123(1): 299-314.

[14] 黎国伟, 王东宁, 叶玉蝶, 等. 急性髓细胞白血病CDX2基因表达及临床意义的探讨[J]. 中华肿瘤防治杂志, 2009, 16(16): 1253-5.

[15] Keenan ID, Sharrard RM, Isaacs HV. FGF signal transduction and the regulation of Cdx gene expression[J]. Dev Biol, 2006, 299(2): 478-88.

[16] Blache P, Van De Wetering M, Duluc I, et al. SOX9 is an intestine crypt transcription factor, is regulated by the Wnt pathway, and represses the CDX2 and MUC2 genes[J]. J Cell Biol, 2004, 166(1): 37-47.

（编辑：黄开颜）